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(71) Applicant *(for all designated States except US)*: THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH [AU/AU]; Royal Parade, Parkville, VIC 3052 (AU).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : BOYD, Andrew, D. [AU/AU]; 34 Baroda Street, Ascot Vale, VIC 3052 (AU). SIMPSON, Richard [AU/AU]; 42 Stanley Street, Richmond, VIC 3121 (AU). WICKS, Ian [AU/AU]; 2/69 Earl Street, Kew, VIC 3101 (AU). WARD, Larry, David [AU/AU]; 46 Nightingale Street, Balaclava, VIC 3183 (AU). WILKINSON, David [AU/AU]; 11/10 Murray Street, West Brunswick, VIC 3055 (AU).

(74) Agents: SLATTERY, John, M. et al.; Davies Collison Cave, 1 Little Collins Street, Melbourne, VIC 3000 (AU).

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(54) Title: A NOVEL RECEPTOR-TYPE TYROSINE KINASE AND USE THEREOF

(57) Abstract

The present invention relates to an isolated receptor-type tyrosine kinase, said tyrosine kinase characterised by, in its naturally occurring form, being reactive to monoclonal antibody III.A4, having an apparent molecular weight of approximately 120-150 kD in its glycosylated form and having an N-terminal amino acid sequence comprising E L I P Q P. More particularly, the present inventino relates to human eph/elk-like kinase (*HEK*).

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A NOVEL RECEPTOR-TYPE TYROSINE KINASE AND USE THEREOF

The present invention relates generally to a novel receptor-type tyrosine kinase and to genetic sequences encoding same.

Tyrosine kinases form an important class of molecules involved in the regulation of growth and differentiation (1). One mode of proof for this role came from the identification of receptors which bind known soluble growth factors. The receptors for epidermal growth factor (EGF) (2), platelet derived growth factor (PDGF) (3) and colony stimulating factor-1 (CSF-1)(4) were all shown to be transmembrane molecules with the cytoplasmic regions encoding a tyrosine kinase catalytic domain. The CSF-1 receptor is homologous to the PDGF receptor in both the catalytic and extracellular domains (1,5). The extra cellular domain of these proteins is distinguished from other tyrosine kinases by the presence of immunoglobulin-like repeats (1,6). Based on structural properties of the kinase domain, the c-kit protein was identified as another member of this family (7). The c-kit gene locus appears to underpin the defects in the congenitally anaemic W/W mouse (8-10). The ligand has now been identified (11-14) as shown to be encoded by the Sl locus. The locus is abnormal in the Steel mouse (15) which has identical defects to the W/W mouse but encodes a normal c-kit gene.

The other line of evidence for a critical role of tyrosine kinase proteins in growth control came from the study of viral oncogenes (16-17). These genes were shown to be directly involved in growth dysregulation by observations of a change in cell growth following introduction of DNA encoding these genes into fibroblasts. All oncogenes have been shown to have close cellular homologues (proto-oncogenes). One of the first identified oncogenes was v-src, the cellular homologue (c-src) is the prototypical representative of the family of cytoplasmic tyrosine kinases which, following myristylation, become associated with the inner leaf of the cell membrane (18). Within the

haemopoietic system a number of lineage-restricted src-like kinases have been defined (19).

The T cell-associated src-like kinase, lck, has been shown to associate independently with both the CD4 and CD8 transmembrane glycoproteins to form a signalling complex (20,21). By contrast, v-erb-B and v-fms, like their cellular homologues the EGF receptor and CSF 1 receptor, respectively, are transmembrane molecules encoding the entire signal transduction machinery in a single polypeptide (1,17).

Detailed analysis of the amino acid sequences of these proteins has revealed conserved structural motifs within the catalytic domains (5). Both tyrosine and serine-threonine kinases have a consensus GXGXXG sequence which is found in many nucleotide binding proteins (5). Other conserved sequence motifs are shared by both types of kinase while others are specific for the tyrosine or the threonine-serine kinase subgroups (5). The tyrosine kinases, while having regions of sequence conservation specific to this family, can be further subdivided according to the structural features of the regions 5' to the catalytic domain (1,4-7). The novel tyrosine kinase of the present invention exhibits the same general characteristics as previously known tyrosine kinases.

In accordance with the present invention, a new receptor-type tyrosine kinase is provided and which is identified as a member of the eph/elk family of tyrosine kinases (22,23). The novel tyrosine kinase receptor is designated *HEK* ("human eph/elk-like kinase"). As the present inventors have identified expression of *HEK* in both pre-B and T cell lines, the receptor molecule of the present invention and/or its ligand is contemplated herein to have particular applicability for use as agents in the in vivo modulation of the production and/or function of pre-B, B and T cells.

Accordingly, one aspect of the present invention provides an isolated receptor-type tyrosine kinase, said tyrosine kinase characterised by, in its naturally occurring form, being reactive to the monoclonal antibody III.A4, having an apparent molecular weight of approximately 120-150 kD in the glycosylated form and having an N-terminal amino acid sequence comprising:

E L I P Q P.

Preferably, the tyrosine kinase has an N-terminal amino acid sequence comprising:

E L I P Q P S N E V N L X D,

wherein X is any amino acid and is preferably L.

More preferably, the tyrosine kinase has an N-terminal amino acid sequence comprising the amino acids:

E L I P Q P S N E V N L X D (S) K X^1 I Q,

wherein X and X^1 are any amino acid and preferably L and T, respectively.

Even more preferably, the tyrosine kinase comprises the amino acid sequence set forth in Figure 1 or any parts or portions thereof, or having an amino acid sequence with at least 30% homology to the amino acid sequence set forth in Figure 1 and having the identifying characteristics of *HEK* More preferably, the degree of homology is at least 40%, still more preferably at least 55, even more preferably at least 70% and still even more preferably greater than 80%.

The hybridoma producing the monoclonal antibody III.A4 was deposited at Public Health Laboratory Service, European Collection of Animal Cell Cultures, Porton Down Salisbury, UK, on 20 June, 1991 under accession number 91061920.

The term "isolated" as used in relation to the tyrosine kinase of the present invention includes a biologically pure preparation comprising at least 20%, preferably at least 40%, more preferably at least 60% and even more preferably at least 80% of the protein relative to other molecules as determined by weight, activity or other convenient means. The term also encompasses any form of the protein not in the naturally occurring state such as, but not limited to, a preparation of membranes containing the protein, a preparation of the protein separate from the membrane or a supernatant fluid comprising said protein. The preparation may be glycosylated, partially unglycosylated or complete unglycosylated or may have a glycosylation pattern altered from what is naturally occurring.

The tyrosine kinase of the present invention is expressed on a number of tumours of human origin. In particular, data are presented herein showing *HEK* expression in human lymphoid tumour cell lines LK63, Lila-1, JM, MOLT4 and HSB-2 and the human epithelial tumour HeLa. One skilled in the art, however, will immediately recognise that similar or homologous kinases may exist on non-tumour cells or on non-human tumours and which have similar properties to the tyrosine kinase of the present invention. For example, the results contained herein show some expression of *HEK* in heart muscle. Accordingly, the present invention extends to a tyrosine kinase functionally and structurally similar in any or all respects to the tyrosine kinase herein described including a kinase of non-tumour origin.

The present invention extends to preparations comprising the naturally occurring form of the tyrosine kinase protein, including any naturally occurring derivative forms thereof, as well as to synthetic and recombinant forms of the protein including any single or multiple amino acid substitutions, deletions and/or insertions to the polypeptide portion of the kinase and to analogues and homologues thereof. Such amino acid alterations to the molecule are examples of recombinant or synthetic mutants and derivatives of the kinase.

Insertions include amino acid and/or carboxyl terminal fusions as well as intra-sequence insertions of single or multiple amino acids. Generally, insertions within the amino acid sequence will be smaller than amino or carboxyl terminal fusions, of the order of say 1 to 4 residues. Insertional amino acid sequence variants are those in which one or more amino acid residues are introduced into a predetermined site in the protein. Deletional variants are characterised by the removal of one or more amino acids from the sequence. Substitutional variants are those in which at least one residue in the sequence has been removed and a different residue inserted in its place. Such subsitutions generally are made in accordance with the following Table 1.

TABLE 1

Original Residue	Exemplary Substitutions
Ala	Ser
Arg	Lys
Asn	Gln; His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
Gly	Pro
His	Asn; Gln
Ile	Leu; Val
Leu	Ile; Val
Lys	Arg; Gln; Glu
Met	Leu; Ile
Phe	Met; Leu; Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

Generally amino acids are replaced by other amino acids having like properties, such as hydrophobicity, hydrophilicity, electronegativity, bulky side chains, etc.

Amino acid substitutions are typically of single residues; insertions usually will be on the order of about 1-10 amino acid residues; and deletions will range from about 1-20 residues. Deletions or insertions preferably are made in adjacent pairs, i.e: a deletion of 2 residues or insertion of 2 residues.

The amino acid variants referred to above may readily be made using peptide synthetic techniques well known in the art, such as solid phase peptide synthesis (Merrifield; J. Am. Chem. Soc., 85: p2149, 1964) and the like, or by recombinant DNA manipulations. Techniques for making substitution mutations at predetermined sites in DNA having known sequence are well known, for example M13 mutagenesis. The manipulation of DNA sequences to produce variant proteins which manifest as substitutional, insertional or deletional variants are well known in the art and are described for example in Maniatis et al (Molecular Cloning: A Laboratory Manual; Cold Spring Harbor Laboratory, 1982).

Other examples of recombinant or synthetic mutants and derivatives of the tyrosine kinase protein of this invention include single or multiple substitutions, deletions and/or additions to any molecule associated with the kinase such as carbohydrates, lipids and/or proteins or polypeptides. Furthermore, it is possible that the tyrosine kinase protein of the present invention is a genetically altered version of a similar protein on normal cells. The present invention, therefore, extends to the tyrosine kinase protein from tumour or non-tumour origin and to all genetically altered forms thereof.

The terms "analogues" and "derivatives" extend to any functional chemical equivalent of the tyrosine kinase protein characterised by its increased stability and/or efficacy in vivo or in vitro. The terms "analogue" and "derivatives" also

extend to any amino acid derivative of the tyrosine kinase protein as described above.

Analogues of *HEK* contemplated herein include, but are not limited to, modifications to side chains, incorporation of unnatural amino acids and/or derivatising the molecule and the use of crosslinkers and other methods which impose conformational constraints on the peptides or their analogues. Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with NaBH4; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6 trinitrobenzene sulphonic acid (TNBS); acylation of arnino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5'-phosphate followed by reduction with $NaBH_4$.

The guanidino group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3- butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation via O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbomoylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hyroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

Modification of the imidaxole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during protein synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids.

Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer groups with n = 1 to n= 6, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bufinctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH). In addition, peptides could be conformationally constrained by, for example, incorporation of C_α and N_α-methylamino acids, introduction of double bonds between C_α and C_β atoms of amino acids and the formation of cyclic peptides or analogues by introducing covalent bonds such as forming an amide bond between the N and C termini, between two side chains or between a side chain and the N or C terminus.

The present invention, therefore, extends to peptides or polypeptides and amino acid and/or chemical analogues thereof having the identifying characteristics of *HEK* as broadly described herein, and/or to regions thereof capable of, or responsible for, its action in transducing signals or in stimulating

cellular responses such as growth and/or differentiation.

Accordingly, reference herein to the receptor-type tyrosine kinase of the present invention includes the naturally occurring molecule, recombinant, synthetic and analogue forms thereof and to any mutants, derivatives and human and non-human homologues thereof. All such kinases are encompassed by the term "*HEK*".

The present invention further extends to the ligand for the novel receptor-type tyrosine kinase described herein and to any agonists and antagonists (e.g. soluble form of the receptor) of the enzyme. Since the tyrosine kinase is an oncogenic protein, antagonists to the receptor are of particular relevance and fall within the scope of the present invention. Such antagonists include antibodies (monoclonal and polyclonal), the enzyme itself in soluble form or otherwise, specific peptides, polypeptides or proteins and carbohydrates, amongst others. These types of antagonists are useful in developing anti-tumour agents where the growth or maintenance of the tumour itself is supported by the tyrosine kinase of the present invention. Accordingly, the addition of an effective amount of an antagonist to the tumour-associated receptor-type tyrosine kinase will inhibit, reduce or otherwise interfere with the receptor activity of the protein and thus prevent, reduce and/or inhibit tumour growth. The present invention, therefore, extends to pharmaceutical compositions comprising one or more antagonists to the tyrosine kinase herein described and one or more pharmaceutically acceptable carriers and/or diluents.

Ligand(s) for *HEK* are capable of being screened for in a number of ways. In one protocol, an expression vector (e.g. AP-TAG-*HEK*) is selected which encodes the entire extracellular region of *HEK* fused to an appropriate reporter molecule like alkaline phosphatase. The fusion protein expressed in cells is recovered from cell supernatants and used to stain (using the reporter molecule) tissue sections using the methods as described by Flanagan and

Leder (39), the disclosure of which is incorporated herein by reference. Once cellular sources of ligand are identified these cells are then used to construct an expression library. If the ligand is cell bound (eg membrane bound), the expression vector (eg. AP-TAG-*HEK*) is used to stain pools to search for positive clones. If the *HEK* ligand is secreted, then another strategy will be required. In this case, supernatants of pools can be used to screen for induction of *HEK* phosphorylation in LK63 or *HEK* transfectants. Alternatively, supernatants from tissues producing *HEK* ligand can be used as a source in affinity purification on columns to which the product of, for example, pEE14-*HEK* is linked as a specific absorbent. The sequence of the purified ligand will be determined and this information used to clone the *HEK* ligand from cDNA libraries.

Another aspect of the present invention is directed to a nucleic acid isolate comprising a sequence of nucleotides encoding the novel receptor-type tyrosine kinase (including its recombinant, synthetic, mutant, derivative, analogue and homologue forms). The nucleic acid sequence may comprise deoxyribonucleotides or ribonucleotides and may exist in single or double stranded form, alone or in combination with a vector or expression vector molecule. The nucleic acid may be naturally occurring RNA or DNA or may be cDNA including complementary forms thereof. The nucleic acid molecule may also contain single or multiple nucleotide substitutions, deletions and/or additions relative to the nucleotide sequence encoding the naturally occurring or recombinant form of the protein. The vectors containing the nucleic acid sequences of the present invention may replicate in eukaryotes and/or prokaryotes and contain promoter sequences capable of expression in one or both of these types of cells. Suitable cells include mammalian, insect, yeast and/or bacterial cells. Particularly preferred cell types include CHO, baculovirus and E. coli cells. The preferred nucleotide sequence comprising *HEK* is set forth in Figure 1. The general techniques of recombinant DNA technology, including isolation of recombinant proteins, are well known and are described for example in Maniatis et al (Supra).

This invention also provides a transgenic cell or cell culture carrying a nucleic acid isolate as described above.

In another aspect, this invention provides a pharmaceutical composition comprising a soluble form of the receptor-type tyrosine kinase as broadly described herein, said composition further comprising one or more pharmaceutically acceptable carriers and/or diluents.

This invention also extends to methods of use of the novel receptor-type tyrosine kinase of this invention and of antagonists to ligands binding to this tyrosine kinase.

In one aspect, this invention extends to a method of ameliorating the effects of interaction or binding between *HEK* and its ligand in a mammal comprising administering to said mammal an effective amount of the antagonist to a ligand binding to the tyrosine kinase of this invention.

The invention also extends to a method of phosphorylating a protein comprising contacting a preparation of said protein with an effective amount of the receptor-type tyrosine kinase of this invention for a time and under conditions sufficient to effect phosphorylation of the protein.

In yet another aspect, the invention provides a method of screening for a ligand bound to tissue or cells to the receptor-type tyrosine kinase of this invention comprising contacting the tyrosine kinase fused to a reporter molecule capable of producing a detectable signal to the tissue or cell sample to be tested for a time and under conditions sufficient for the fused tyrosine kinase to bind to a ligand on said tissue or cells and then detecting the reporter molecule.

The invention further provides a method of screening for a soluble ligand to the receptor-type tyrosine kinase of this invention comprising contacting a sample to be tested with a cell line capable of expressing the tyrosine kinase and screening for phosphorylation in said cell line.

One skilled in the art will, however, immediately recognise that a variety of mutations, derivatives or chemical alternations can be made to the sequence to encode, for example, the analogues and derivatives disclosed above. The present invention also extends to short nucleic acid molecules which can act as nucleic acid probes to screen for the presence of the *HEK* gene or mutations therein.

The present invention is further described with reference to the following non-limiting Figures and Examples.

In the Figures:

Figure 1 is a representation showing nucleotide sequence and deduced amino acid sequence of *HEK* coding sequence with partial 3' and 5' untranslated sequence. Numbers at right indicate positions of nucleotides and numbers above amino acids refer to amino acid sequence. A single underline indicates the presumed signal peptide. Double underline indicates the presumed transmembrane region. Dashed overline indicates identity between the predicted amino acid sequence and the sequence obtained from purified *HEK* protein. Triangles indicate potential sites for N-linked glycosylation within the extra-cellular domain. Dots indicate the putative ATP-binding site. The diamond indicates a putative autophosphorylation site. Asterisks indicate stop codons.

Figure 2 is a representation showing protein sequence alignment of *HEK* with elk, a related gene within the eph/elk family. Alignment was performed using the GAP programme. Amino acid positions are numbered on the right. Dots in the sequence indicate gaps introduced to optimise the alignm,ent. Dashes indicate identity between amino acids. Asterisks indicate stop codons. Dots above the line of amino acids indicates residues contributing to the two repeats of homology with fibronectin type III, within the C-terminal regions of the extracellular domains. Triangles above the line of amino acids highlight conserved cyseine residues within the N-terminal region.

Figure 3 is a photographic representation showing expression of *HEK* in COS cells. The *HEK* 4.5 kb cDNA clone was subcloned into the expression vector CDM8. COS cells were transfected with this construct using DEAE-dextran/chloroquine and DMSO. Two days after transfection cells were stained in situ with the IIIA4 MAb followed by FITC-conjugated sheep anti-mouse Ig and photographed under light microscopy (panel A), or fluorescence microscopy (panel B). Magnification X400.

Figure 4 is a photographic representation of Northern blot analysis of *HEK* expression in cell lines. Poly (A) + RNA from hurnan cell lines was fractionated on an agarose/formaldehyde gel and transferred onto Hybond-C extra membrane. The filter was hybridised with the *HEK* 4.5 kb cDNA (upper panel). The same filter was hybridised with GAPDH as a quantitative control (lower panel). REH, NALM-1 and FAKEM are pre-B leukaemic cell lines. BALL-1 is an early B leukaemic cell line. RAMOS is a mature B leukaemic cell line. HSB-2, HPB-ALL and JM are T leukaemic cell lines.

Figure 5 is a photographic representation showing Northern blot analysis of *HEK* expression in cell lines. Poly A^+ RNA from human cell lines was probed for *HEK* expression as above. Molt 4 is an immature T cell line. RC2a, HL60 and U937 are myelomonocytic cell lines. In this experiment, RNA was

extracted from HL60 and U937 after treatment of cells with tetra decannoyl phorbol myristic acetate (TPA), an activator of protein kinase C. U266 is a mature B cell line.

Figure 6 is a photographic representation showing Northern analysis of *HEK* expression in adult post mortem tissues. A multiple tissue Northern blot was purchased commercially and probed for *HEK* expression under conditions suggested by the manufactuerer (Clontech). The 1.3kb band in pancreas is too small to represent a transcript for a secreted form of *HEK* and is probably due to cross hybridisation.

Figure 7 is a photographic representation showing Southern blot analysis of *HEK* in cell lines and normal human peripheral blood cell DNA. Samples were digested with Hind III (lanes 1-3) or Bam HI (lanes *4-6*), run on a 1% agarose gel and transferred to Zetaprobe membrane. The membrane was hybridised with a 1.1 kb fragment of *HEK*, extending from nucleotides 1,109 to 2,241 (see Fig 1). Lanes 1 and 4, normal peripheral blood; Lanes 2 and *5*, LK63 cells; lanes 3 and 6, LK63/CD20+ cells.

Figure 8 is a photographic representation showing in situ hybridisation. The ~1.1kb *HEK* PCR product referred to above was nick translated with biotin-14-dATP and hybridised in situ at a probe concentration of 5 ng/µl to metaphases from two normal males. Chromosomes were stained before analysis with both propidium iodide (as counterstain) and DAPI (for chromosome identification).

Figure 9 is a graphical representation showing a hydropathy analysis (span length: 25) of the predicted translational product of the *HEK* 4.5 cDNA. The Y axis indicates a hydropathy index, with hydrophobic residues appearing above the origin and hydrophilic residues below. The AAs comprising the translated product of the *HEK* cDNA are numbered along the X axis from 1-983.

EXAMPLE

1. Materials and Methods

Cell lines, Mab III4 *HEK* protein structure and function

The LK63 and LK63/CD20+ cell lines were derived from a child with acute lymphoblastic leukaemia. LK63/CD20+ is a tetraploid variant of LK63, which arose spontaneously in vitro and has enhanced *HEK* expression. In contrast to the parental cell line, LK63/CD20+ expresses CD20. These lines have cytogenetic features of pre-B cell leukaemia and have not been transformed with Epstein-Barr virus (24). JM and HSB-2 are CD8 +, human T cell leukaemic cell lines.

The IIIA4 Mab was generated against the LK63 cell line and recognised a 135kD, cell surface molecule *(HEK)* with in vitro kinase activity expressed by LK63, LK63/CD20+ and JM (25).

The IIIA4 Mab was used to purify *HEK* antigen for amino acid sequencing (25). The amino acid sequences obtained were as follows, where doubtful residues are bracketed and unidentified residues are marked X: N terminus- ELIPQPSNEVNLXD(S)KXIQ; internal- GYRLPPPMDCPAALYQLMLDC.

LK63 cDNA library construction and screening

A random primed cDNA library was constructed in λgtl0 (Amersham) using 5ug of poly A+ selected mRNA from LK63/CD20+ cells. A degenerate oligonucleotide was designed on the basis of the internal (3') *HEK* protein sequence. The neutral base inosine was included at positions of high codon degeneracy (26). The 51 mer:

TACCGICTICCICCICCIATGGACTGCCCIGCIGCICTITACCAACTIATG
T T T G

was end labelled using γ32P-deoxyadenosine triphosphate (ATP) and polynucleotide kinase, followed by separation on a G25 Sephadex column as previously described (27). Approximately 250,000 plaques were screened in 2xSSC (SSC = 0.15M NaCl, 0.015M sodium citrate) hybridisation buffer at 37°, as previously described *(27)*. Washes were performed in 2xSSC/0.1% w/v sodium dodecyl sulphate (SDS) at 42-55°. The signal from one duplicating plaque persisted following 55° washes. The DNA from this plaque contained an insert of 2.5 kb *(HEK*2.5). *HEK*2.5 was labelled with α^{32}P-ATP (Amersham random primer kit) for Northern blot analysis of LK63 cells. The polymerase chain reaction (PCR) was performed using *HEK*2.5 and oligonucleotide primers based on conserved motifs within the catalytic domain of PTKs and the 3' amino acid *HEK* sequence, as previously described (28). *HEK*2.5 was labelled with α^{32}P-ATP (as above) and used to rescreen the random primed LK63 cDNA library in 2xSSC hybridisation buffer at 65°. Thirty two duplicating positives were isolated and screened by hybridisation with a degenerate oligonucleotide based on the N terminal *HEK* protein sequence. A 4.5 kb *HEK* clone (*HEK*4.5) which hybridised with the N terminal oligonucleotide was chosen for complete characterisation.

DNA sequencing and analysis of *HEK* cDNA

*HEK*4.5 was subcloned into pGEM7 which had been digested with EcoRI and treated with calf intestinal phosphatase. Double stranded DNA was purified on a caesium chloride gradient and used as the template in dideoxy chain termination sequence reactions (29). Sense and antisense oligonucleotide primers were used to complete sequencing with T7 DNA polymerase (Promega). Protein sequence alignment was performed using the GAP programme (University of Wisconsin, Genetics Computer Group).

Expression of *HEK* in COS cells

The *HEK* 4.5 EcoRI insert was blunt ended with Klenow DNA polymerase 1 and dATP plus dTTP, followed by ligation to BstXI adaptors. The adapted insert was ligated to BstXI digested CDM8 (30). Sense and antisense constructs were prepared and transfected into COS cells using DEAE-dextran/chloroquine with dimethyl sulphoxide (DMSO) (17). Two days post-transfection, COS cells were stained with IIIA4 followed by fluorescein isothiocyanate conjugated (FITC)-conjugated sheep anti-mouse immunoglobulin (Ig) (Silenus) and examined under a fluorescence microscope.

Northern and Southern blot analysis of cell lines

Poly A+ selected mRNA was isolated as previously described (31) and fractionated on a 1% formaldehyde agarose gel prior to transfer onto a HybondC extra membrane (Amersham). Filters were probed with *HEK* 4.5 and subsequently with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) insert as a control. DNA was prepared by lysis with guanidine hydrochloride (32), transferred to Zetaprobe membranes and hybridised under conditions suggested by the manufacturer (Bio-Rad). In order to minimise cross hybridisation with other tyrosine kinases in Southern analysis of genomic DNA, PCR was used to generate a 1.1 kb *HEK* probe which spans a less highly conserved region of the molecule (nucleotides 1,109 to 2,241, Fig.1). The autoradiogram of the Southern blot was digitised using the MacScan programme on a Macintosh IIx computer.

Scatchard analysis of IIIA4 binding to cell lines

Binding of ^{125}I labelled IIIA4 to cell lines was performed in competition with unlabelled IIIA4 as previously described (33).

Protein analysis

The *HEK* protein was subjected to hydrophobicity analysis as described by Kyte and Doolittle (40). The results are shown in Figure 9.

Oligos to construct expression vectors encoding variants of the extracellular domain of *HEK*

Primer *HEK*S'/92 has the following sequence:

```
     Bam H1  Eco R1
5    ┌─────┐ ┌─────┐
GTAGGGATCCGAATTCTGCACCAGCAACATG
                └──────────────┘
```

The BamH1 and Eco R1 sites are indicated above the sequence and the underlined portion corresponds to positions 86 to 102 of the sequence set forth in Figure 1.

Primer *HEK*/EE14/92 has the sequence:

```
15     Bam H1
       ┌─────┐
GTAGGGATCCTACACTTGGCTACTTTCA
          └┘ └──────────────┘
        Stop Codon
```

20

The underlined portion after the stop codon is the reversed and complemented sequence of nucleotide 1710-1725 of Figure 1.

```
  Bam H1
25 ┌─────┐
GCGGATCCTTGCCTACTTTCACCA,
       └──────────────┘
```

The underlined sequence when reversed and complemented corresponds to 30 1708-1723 of the sequence in Figure 1 and does not contain the stop codon permitting read through from the BamH1 site.

PCR Conditions

PCR was performed with Taq polymerase under standard conditions using CsCl purified pGEM7-*HEK*, which contains the full length *HEK* cDNA, as a template. Cycle times and temperatures:

60' at 97°C
60' at 55°C
90' at 73°C

the reaction was carried out for ten cycles.

1. The 1.7 kb PCR product of the *HEK*5'/92 and *HEK*/EE14/92 was purified using Geneclean, digested with Eco R1 and BamH1 and cloned between the Eco R1 and Bcl I site of pEE14 (obtained from Celltech, Berkshire, UK).

Analysis showed the predicted 1.7 kb insert in the clones which were designated "pEE14-*HEK*".

2. The 1.7kb PCR product of 5'*HEK*5'/92 and *HEK*/TAG/3' was digested with BamH1, cloned into BglII site of AP-Tag-1, Flanagan & Leder (39). Using SnaB1, the sense of the clones could be determined to fused clones with the correct orientation. The resulting clones were designated AP-TAG-*HEK*

Expression

pEE14-*HEK* was transfected into CHO cells and lines selected with methionine sulfoxime.

AP-TAG-*HEK* was transfected into 3T3 cells with pSV2 neo and clones selected with G418.

EXAMPLE

2. *HEK*

Isolation and characterisation of cDNA clones for *HEK*

One duplicating signal was obtained from screening approximately 250,000 plaques of an LK63-derived λgtl0 cDNA library under relaxed conditions with a degenerate 51 mer oligonucleotide. This plaque contained a 2.5 kb insert *(HEK*2.5) which hybridised with a single 5.5-6.0 kb mRNA species in Northern blot analysis of cell lines expressing *HEK* i.e. LK63 and JM. PCR using *HEK* 2.5 and oligonucleotide primers based on conserved motifs within the catalytic domains of tyrosine kinases *(28)*, gave DNA products of the appropriate size. These results indicated *HEK* 2.5 was truncated at the 5' end. *HEK* 2.5 was used to re-screen the library under more stringent conditions and a 4.5 kb *HEK* (*HEK* 4.5) clone isolated. This clone hybridised with a degenerate oligonucleotide based on the N terminal protein sequence and produced DNA bands of the predicted sizes in PCR reactions using the primers referred to above. These data indicated the 4.5 kb clone probably contained the complete *HEK* coding region.

The sequence of the coding region for *HEK*, together with partial 3' and 5' untranslated sequence, is shown in Figure 1. An open reading frame of 2,952 nucleotides extends from the initiation methionine at position 100 to the first termination codon at position 3051. Translation of the cDNA results in a predicted protein of 983 amino acids (AAs). There is identity between the AAs obtained by sequencing of purified *HEK* protein and the predicted AA product of the cDNA clone (see Fig. 1). The predicted molecular weight of the translated protein (minus the putative signal peptide) is 92.8 kD. This is in good agreement with previous results demonstrating a core protein of approximately 95 kD in both tunicamycin- and endoglycosidase-treated LK63 cells (25). The predicted protein product of the *HEK* cDNA clone has the features of a type 1a integral membrane protein (35). Two predominantly

hydrophobic regions indiciate a putative signal peptide (AAs 1-20) and a transmembrane segment (AAs 542-565). The extracellular domain of 521 AAs contains five possible sites for N linked glycosylation. The N terminal region (AAs 21-376) of the extracellular domain is rich in cysteine residues. The C-terminal region (AAs 326-511) of the extracellular domain contains two repeats homologous to those found in fibronectin type III (36). The cytoplasmic domain (AAs 566-983) of *HEK* contains a typical ATP binding site (GXGXXG) at AA positions 628-633 and a putative autophosphorylation site (E/DXXYXX) at position 779.

Protein sequence alignment shows a high degree of homology between *HEK* and eph, elk, eck, eek and erk in the catalytic domains. *HEK* has the following overall protein sequence homology with each of the three sequenced members of the eph RTK family: (chicken) CEK 56.4%, (rat) elk 56.1%; (rat) eck 50.6%; (human) eph 42.3%. Protein sequence alignment between *HEK* and a close relative ELK is shown in Figure 2. The homology between these molecules is greatest within the catalytic domains. Outside the catalytic domains, numerous short motifs which may be of structural or functional significance, are conserved between *HEK*, eph, elk and eck, particularly towards the N terminus. There is strict conservation of the number and spatial arrangement of cysteine residues within the extracellular domains of *HEK*, eph, elk and eck (34). These cytokine residues cluster within the N terminal portion of the extracellular domains (36). The C terminal regions of the extracellular domains contain repeats which are homologous to those found in fibronectin type III (36). *HEK* has a cysteine in the C terminal tail (AA928), rather than the tyrosine which is conserved in this position between other members of the EPH/ELK family. This may be of significance in that phosphorylation of C terminal tyrosine residues can regulate tyrosine kinase activity (37). However *HEK* has a C terminal tyrosine at position 937, which also appears to be in a better context for autophosphorylation (38).

Transfection and expression of *HEK* in COS cells

To demonstrate that the cDNA clone isolated did indeed encode the molecule recognised by the IIIA4 Mab, *HEK* 4.5 was subcloned into the expression vector CDM8 and transfected into COS cells in both sense and antisense orientations. As shown in Figure 3, COS cells transfected with *HEK* in the sense orientation stained specifically with IIIA4, confirming that the cDNA clone contains the full coding sequence and is identical to the molecule recognised by IIIA4. COS cells transfected with *HEK* in the antisense orientation did not stain with IIIA4.

Expression of *HEK* in human lymphoid cell lines

Cell surface staining with IIIA4 revealed a highly restricted pattern of *HEK* expression on LK63 - a pre B cell line, and JM - a T cell line. To further explore the expression of *HEK*, Northern blot analysis was performed with *HEK* 4.5 (Figures 4 to 6). A single 5.5-6.0 kb band was seen in both LK63 and JM cells. However there was a less intense band of the same size in another T cell line - HSB-2 - which did not stain with IIIA4. Other cell lines in which *HEK* transcripts were detected include Lila-1, MOIT4 and HeLa. There were no *HEK* transcripts detected in a range of other cell lines although a weak band was seen in heart muscle (Figure 6). The number of *HEK* molecules was determined on HSB-2, LK63/CD20+ and other cells using Scatchard analysis of IIIA4 MAb binding. The LK63/CD20+ cells had approximately 15,000 sites per cell and JM cells had 9,500 sites per cell. In contrast, HSB-2 had approximately 1,070 sites per cell, which is too low for detection by immunofluorescence against the autofluorescence background of this cell line. The affinity constants for antibody binding were in the range of 2.5-4.0 x 10^9. Raji and K562 cells showed no detectable antibody binding above background. Tables 1 and 2 summarise the phenotype of *HEK* expression cell lines.

Southern blot analysis

To investigate the basis for overexpression of *HEK* in the lymphoid tumour cell line LK63, Southern analysis of genomic DNA was performed (Figure 7). A 1.1 kb fragment covering a less conserved region of *HEK* (see above), was used as a probe in order to minimise background arising from conserved regions of the catalytic domains of related tyrosine kinase molecules. Compared with normal peripheral blood mononuclear cell DNA, there is no apparent amplification or rearrangement of the *HEK* gene in the LK63 or LK63/CD20+ tumour cell lines.

Chromosomal assignment of *HEK*

HEK cDNA was used as a probe to locate the position of the *HEK* gene within the normal human chromosome complement. Chromosomal assignment was performed in two ways - by in situ hybridisation and by Southern analysis of somatic cell hybrids. Thirty normal male metaphases were examined for a fluorescent signal. Twenty four of these metaphases showed signal on one or both chromatids of chromosome 3 in the region of 3cen→3p12.1. 85% of this signal was at 3p11.2 (Figure 8). There were a total of nine non-specific background dots observed in these 30 metaphases. Similar results were obtained from the hybridisation to the second male. Southern blot analysis of the hybrid cell panel showed hybridisation of the *HEK* probe only to hybrids containing material from human chromosome 3. Bands of 5.2, 4.8, 4.3, 2.4 and 1.9 kb were obtained from the Hind III digest and bands of 4.3, 3.2 and 1.9kb were obtained from the Taq1 digest. The hybrid cell panel used represents the entire human genome except for chromosomes 2, 6q, 8, 11p and Y. The results from both techniques thus localised the *HEK* gene to chromosome 3 and in situ hybridisation analysis positioned this more precisely to 3p11.2. This region was not cytogenetically abnormal in *HEK*-positive tumours. Similarly, there was no isolated change in the copy number of chromosome 3 in *HEK*-positive cell lines and no isochromosome formation involving chromosome 3.

TABLE 1 **Phenotype of *HEK*-positive human lymphoid cell lines.**

The phenotype of *HEK*-positive cell lines was determined by staining for T and B cell markers followed by FACS analysis. + weakly positive, + + positive, + + + strongly positive.

	IILA4	IgM	CD19	CD20	CD1	CD2	CD3	CD4	CD7	CD8
LK63	++	+	+	-	-	-	-	-	-	-
LK63T	+++	++	++	++	-	-	-	-	-	-
Lila-1	-	+	++	-	-	-	-	-	-	-
HSB-2		-	-	-	-	-	+		++	
JM	+	-	-	-	+	-	+	++	++	++
Molt4	-	-	-	-	+	++	-	-	++	+

TABLE 2 Summary of *HEK* expression in human cell lines

HEK-positive cell lines were characterised using a combination of cell surface staining, Northern blot analysis and Scatchard analysis. + weakly positive, + + positive, + + + strongly positive. NT, not tested.

Line	Lineage	IILA4/IF	Receptors/cell	RNA
LK63	Pre-B	++	15,000	++
LK63T	Pre-B	+++	NT	+++
Lila	Pre-B	-	NT	+
JM	T cell	++	9,500	++
HSB-2	T cell	-	1,100	+
Molt 4	T cell	-	NT	+
HeLa	Cervical	-	NT	+

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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CLAIMS:

1. An isolated receptor-type tyrosine kinase, said tyrosine kinase characterised by, in its naturally occurring form, being reactive to monoclonal antibody III.A4, having an apparant molecular weight of approximately 120-150 kD in its glycosylated form and having an N-terminal amino acid sequence comprising:

E L I P Q P

2. The tyrosine kinase according to claim 1 having an N-terminal amino acid sequence comprising:

E L I P Q P S N E V N L X D (S) K X' I Q

wherein X and X' are any amino acid.

3. The tyrosine kinase according to claim 2 wherein X and X' are L and T, respectively.

4. An isolated receptor-type tyrosine kinase having the amino acid sequence substantially as set forth in Figure 1 or any parts or portions thereof, or having an amino acid sequence with at least 30% homology to the sequence set forth in Figure 1 and having the identifying characteristics of a human eph/elk-like kinase (*HEK*).

5. The tyrosine kinase according to any one of claims 1 to 4 in recombinant or synthetic form.

6. A nucleic acid isolate comprising a sequence of nucleotides encoding, or complementary to a sequence encoding, the tyrosine kinase according to claim 5.

7. A transgenic cell or cell culture carrying the nucleic acid isolate according to claim 6.

8. A transgenic cell or cell culture according to claim 7 which is a mammalian, insect, yeast or bacterial cell.

9. A transgenic cell or cell culture according to claim 8, wherein the cell is a CHO, baculovirus or E. coli cell.

10. An antagonist to a ligand binding to the tyrosine kinase of claim 1.

11. A pharmaceutical composition comprising a soluble form of a receptor-type tyrosine kinase, said tyrosine kinase characterised by, in its naturally occurring form, being reactive to monoclonal antibody III.A4, having an apparent molecular weight of approximately 120-150kD in its glycosylated form and having an N-terminal amino acid sequence comprising:

E L I P Q P,

said composition further comprising one or more pharmaceutically acceptable carriers and/or diluents.

12. The pharmaceutical composition according to claim 11 wherein the tyrosine kinase has an N-terminal amino acid sequence comprising:

E L I P Q P S N E V N L X D (S) K X' I Q

wherein X and X' are any amino acid.

13. The pharmaceutical composition according to claim 11 wherein X and X' are L and T, respectively.

14. The pharmaceutical composition according to claim 11 wherein the tyrosine kinase has an amino acid sequence substantially as set forth in Figure 1 or any parts or portions thereof, or having an amino acid sequence with at least 30% homology to the sequence set forth in Figure 1 and having the identifying characteristics of a human eph/elk-like kinase (*HEK*).

15. The pharmaceutical composition according to any one of claims 11 to 14 wherein the tyrosine kinase is in recombinant or synthetic form.

16. A method of ameliorating the effects of interaction or binding between *HEK* and its ligand in a mammal comprising administering to said mammal an effective amount of the antagonist according to claim 10.

17. The method according to claim 16 wherein the antagonist is a soluble form of *HEK* or a derivative or part thereof.

18. The method according to claim 17 further comprising the administration sequentially or simultaneously of another active compound.

19. The method according to claim 18 wherein the other active compound is a cytokine or an anticancer agent.

20. A method of phosphorylating a protein comprising contacting a preparation of said protein with an effective amount of the receptor-type tyrosine kinase of claim 1 for a time and under conditions sufficient to effect phosphorylation of the protein.

21. The method according to claim 19 wherein the receptor-type tyrosine kinase is *HEK*

22. A method of screening for a ligand bound to tissue or cells to the receptor-type tyrosine kinase of claim 1 comprising contacting the tyrosine kinase fused to a reporter molecule capable of producing a detectable signal to the tissue or cell sample to be tested for a time and under conditions sufficient for the fused tyrosine kinase to bind to a ligand on said tissue or cells and then detecting the reporter molecule.

23. The method according to claim 22 wherein the reporter molecule is alkaline phosphatase.

24. The method according to claim 22 or 23 wherein the tyrosine kinase is encoded by AP-TAG-*HEK*

25. A method of screening for a soluble ligand to the receptor-type tyrosine kinase of claim 1 comprising contacting a sample to be tested with a cell line capable of expressing the tyrosine kinase and screening for phosporylation in said cell line.

26. The method according to claim 25 wherein the cell line is LK63 or a *HEK* transformant cell line.

27. The method according to claim 25 or 26 wherein the sample is a supernatant fluid.

28. The method according to claim 25 alternatively comprising passing the sample through an affinity column having the tyrosine kinase immobilised thereon.

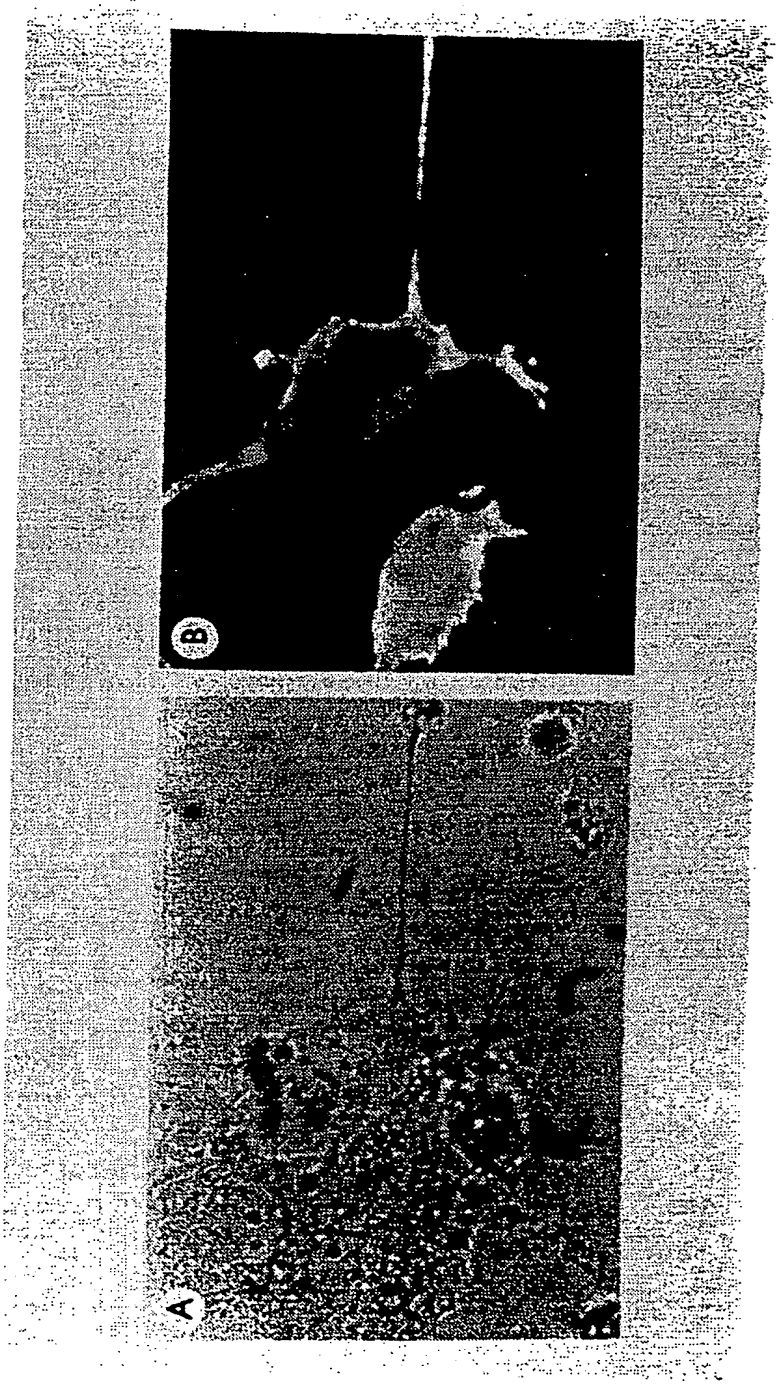

FIG 3B

FIG 3A

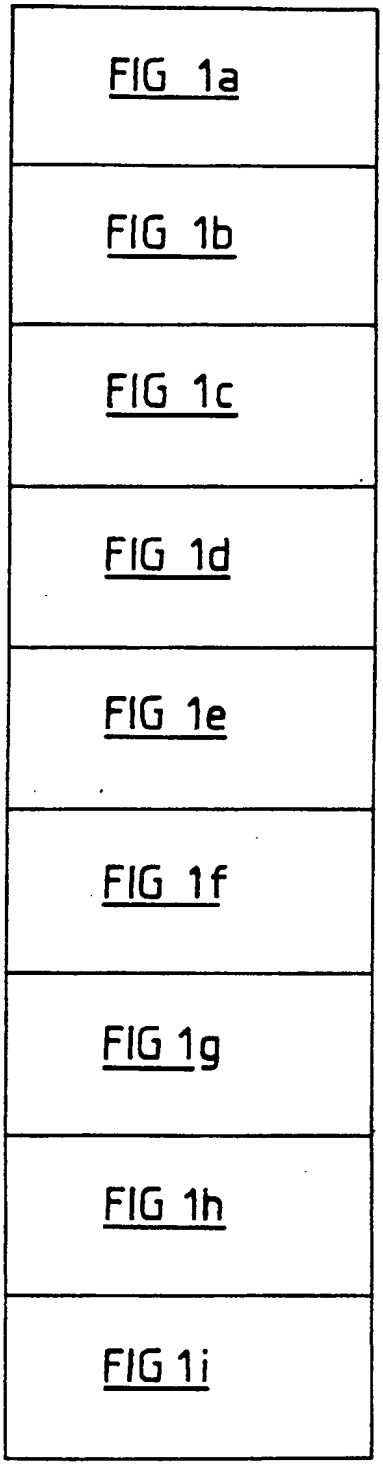

FIG 1

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```
                *
CATGGATGGTAACTTCTCCAGCAATCAGAGCGCTCCCCCTCACATCAGTGGCATGCTTCATGGA

                                    1.
                                    M  D  C
GATATGCTCCTCTCACTGCCCTCTGCACCAGCAAC ATGGATTGT                          108

                    10                                  20 ------------
 Q  L  S  I  L  L  L  L  S  C  S  V  L  D  S  F  G  E  L  I  P
CAGCTCTCCATCCTCCTCCTTCTCAGCTGCTCTGTTCTCGACAGCTTCGGG GAACTGATTCCGC

-----------------------------------------
Q  P  S  N  E  V  N  L  L  D  S  K  T  I  Q
AGCCTTCCAATGAAGTCAATCTACTGGATTCAAAAACAATTCAA                        216

40                                  50                          60
 G  E  L  G  W  I  S  Y  P  S  H  G  W  E  E  I  S  G  V  D  E
GGGGAGCTGGGCTGGATCTCTTATCCATCACATGGGTGGGAAGAGATCAGTGGTGTGGATGAAC

                                70
H  Y  T  P  I  R  T  Y  Q  V  C  N  V  M  D
ATTACACACCCATCAGGACTTACCAGGTGTGCAATGTCATGGAC                        324

            80                                  90
 H  S  Q  N  N  W  L  R  T  N  W  V  P  R  N  S  A  Q  K  I  Y
CACAGTCAAAACAATTGGCTGAGAACAAACTGGGTCCCCAGGAACTCAGCTCAGAAGATTTATG
```

FIG 1a

```
                100                                 110
V  E  L  K  F  T  L  R  D  C  N  S  I  P  L
TGGAGCTCAAGTTCACTCTACGAGACTGCAATAGCATTCCATTG

                    120                                 130
 V  L  G  T  C  K  E  T  F  N  L  Y  Y  M  E  S  D  D  D  H  G
GTTTTAGGAACTTGCAAGGAGACATTCAACCTGTACTACATGGAGTCTGATGATGATCATGGGG

                140
V  K  F  R  E  H  Q  F  T  K  I  D  T  I  A
TGAAATTTCGAGAGCATCAGTTTACAAAGATTGACACCATTGCA                    540

          150                                 160
 A  D  E  S  F  T  Q  M  D  L  G  D  R  I  L  K  L  N  T  E  I
GCTGATGAAAGTTTCACTCAAATGGATCTTGGGGACCGTATTCTGAAGCTCAACACTGAGATTA

  170                                 180
R  E  V  G  P  V  N  K  K  G  F  Y  L  A  F
GAGAAGTAGGTCCTGTCAACAAGAAGGGATTTTATTTGGCATTT                    648

                    190                                 200
 Q  D  V  G  A  C  V  A  L  V  S  V  R  V  Y  F  K  K  C  P  F
CAAGATGTTGGTGCTTGTGTTGCCTTGGTGTCTGTGAGAGTATACTTCAAAAAGTGCCCATTTA

                210
T  V  K  N  L  A  M  F  P  D  T  V  P  M  D
CAGTGAAGAATCTGGCTATGTTTCCAGACACGGTACCCATGGAC                    756
```

FIG 1b

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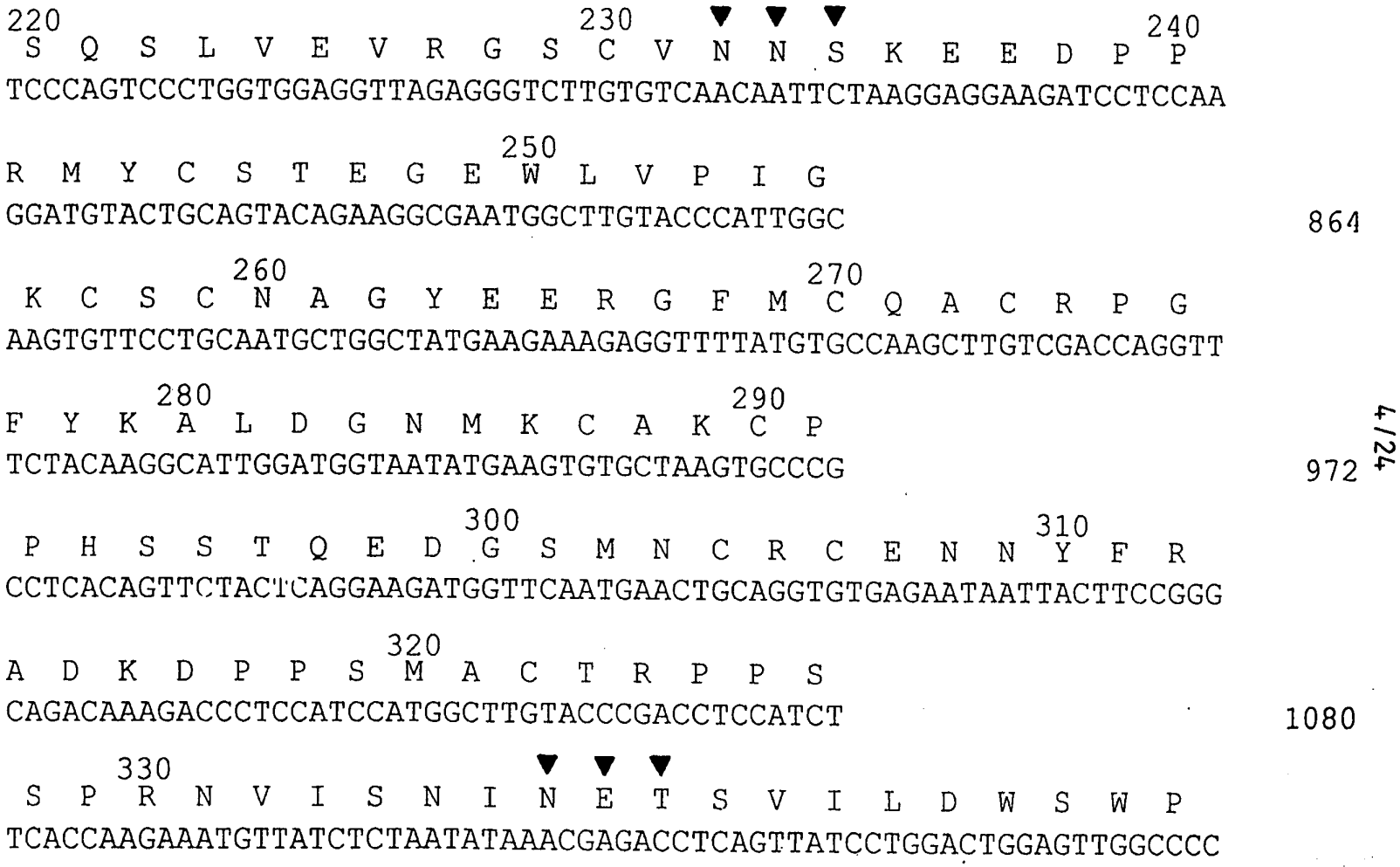

FIG 1c

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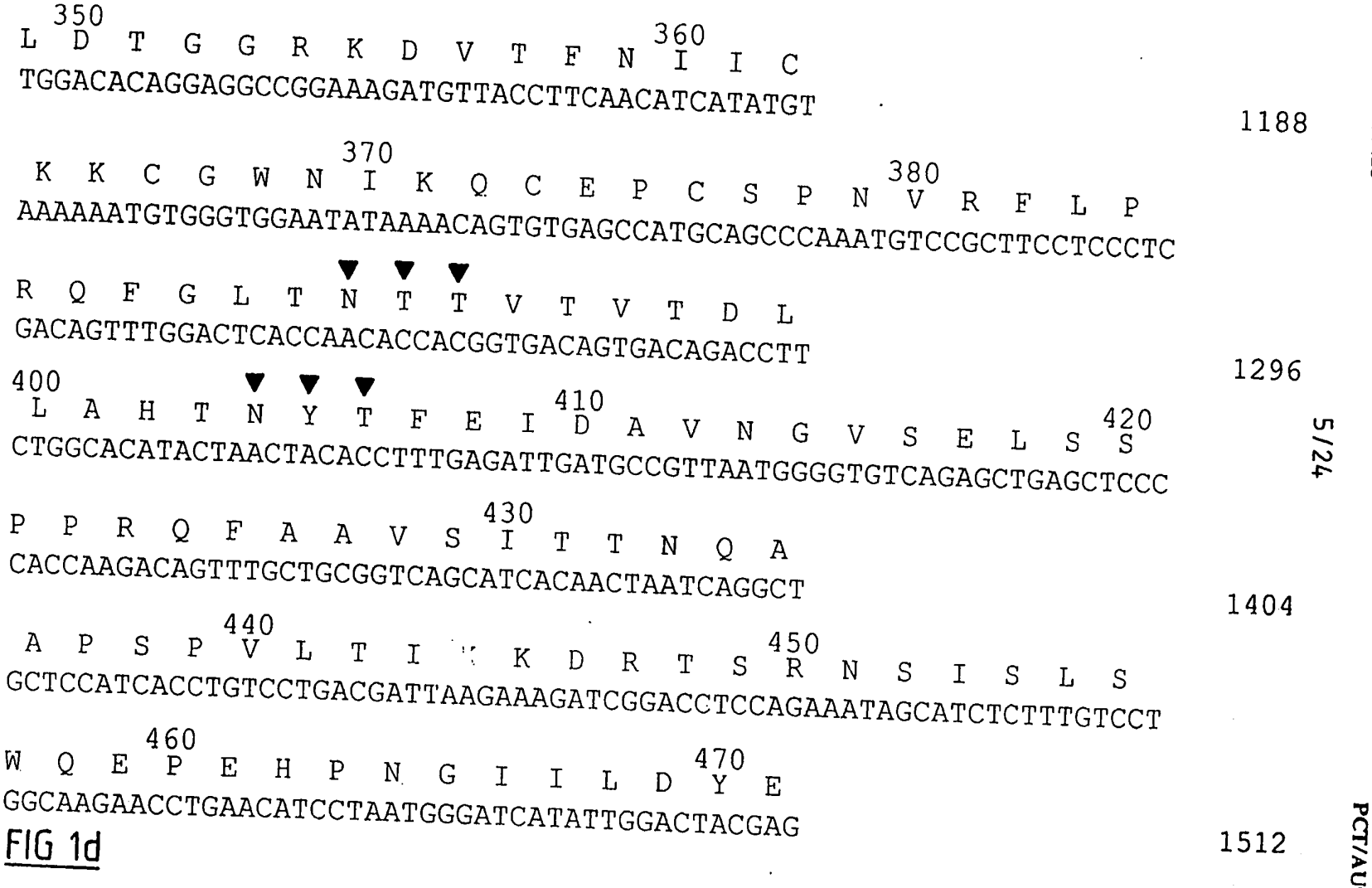

FIG 1d

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                                480                                490
 V  K  Y  Y  E  K  Q  E  Q  E  T  S  Y  T  I  L  R  A  R  G  T
GTCAAATACTATGAAAAGCAGGAACAAGAAACAAGTTATACCATTCTGAGGGCAAGAGGCACAA

▼  ▼  ▼              500
N  V  T  I  S  S  L  K  P  D  T  I  Y  V  F
ATGTTACCATCAGTAGCCTCAAGCCTGACACTATATACGTATTA                      1620

     510                                520
 Q  I  R  A  R  T  A  A  G  Y  G  T  N  S  R  K  F  E  F  E  T
CAAATCCGAGCCCGAACAGCCGCTGGATATGGGACGAACAGCCGCAAGTTTGAGTTTGAAACTA

  530                                540
S  P  D  S  F  S  I  S  G  E  S  S  Q  V  V
GTCCAGACTCTTTCTCCATCTCTGGTGAAAGTAGCCAA GTGGTC                     1728

                    550                                560
 M  I  A  I  S  A  A  V  A  I  I  L  L  T  V  V  I  Y  V  L  I
ATGATCGCCATTTCAGCGGCAGTAGCAATTATTCTCCTCACTGTTGTCATCTATGTTTTGATTG

                570
G  R  F  C  G  Y  K  S  K  H  G  A  D  E  K
GGAGGTTCTGTGGCTATAAGTCAAAACATGGGGCAGATGAAAAA                      1836

580                                590                                600
 R  L  H  F  G  N  G  H  L  K  L  P  G  L  R  T  Y  V  D  P  H
AGACTTCATTTTGGCAATGGGCATTTAAAACTTCCAGGTCTCAGGACTTATGTTGACCCACATA
```

FIG 1e

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```
                                610
T  Y  E  D  P  T  Q  A  V  H  E  F  A  K  E
CATATGAAGACCCTACCCAAGCTGTTCATGAGTTTGCCAAGGAA                          1944

          620                    •     •     •
 L  D  A  T  N  I  S  I  D  K  V  V  G  A  G  E  F  G  E  V  C
TTGGATGCCACCAACATATCCATTGATAAAGTTGTTGGAGCAGGTGAATTTGGAGAGGTGTGCA

       640                                650
S  G  R  L  K  L  P  S  K  K  E  I  S  V  A
GTGGTCGCTTAAAACTTCCTTCAAAAAAAGAGATTTCAGTGGCC                          2052

                         660                                670
 I  K  T  L  K  V  G  Y  T  E  K  Q  R  R  D  F  L  G  E  A  S
ATTAAAACCCTGAAAGTTGGCTACACAGAAAAGCAGAGGAGAGACTTCCTGGGAGAAGCAAGCA

                      680
I  M  G  Q  F  D  H  P  N  I  I  R  L  E  G
TTATGGGACAGTTTGACCACCCCAATATCATTCGACTGGAAGGA                          2160

     690                                700
 V  V  T  K  S  K  P  V  M  I  V  T  E  Y  M  E  N  G  S  L  D
GTTGTTACCAAAAGTAAGCCAGTTATGATTGTCACAGAATACATGGAGAATGGTTCCTTGGATA

  710                                720
S  F  L  R  K  H  D  A  Q  F  T  V  I  Q  L
GTTTCCTACGTAAACACGATGCCCAGTTTACTGTCATTCAGCTA                          2268
```

FIG 1f

```
                    730                                 740
 V  G  M  L  R  G  I  A  S  G  M  K  Y  L  S  D  M  G  Y  V  H
CTGGGGATGCTTCGAGGGATAGCATCTGGCATGAAGTACCTGTCAGACATGGGCTATGTTCACC

               750
R  D  L  A  A  R  N  I  L  I  N  S  N  L  V
GAGACCTCGCTGCTCGGAACATCTTGATCAACAGTAACTTGGTG                    2376

760                                 770                    ◆
 C  K  V  S  D  F  G  L  S  R  V  L  E  D  D  P  E  A  A  Y  T
TGTAAGGTTTCTGATTTCGGACTTTCGCGTGTCCTGGAGGATGACCCAGAAGCTGCTTATACAA

T  R  G  G  K  I  P  I  R  W  T  S  P  E  A
CAAGAGGAGGGAAGATCCCAATCAGGTGGACATCACCAGAAGCT                    2484

          800                                 810
 I  A  Y  R  K  F  T  S  A  S  D  V  W  S  Y  G  I  V  L  W  E
ATAGCCTACCGCAAGTTCACGTCAGCCAGCGATGTATGGAGTTATGGGATTGTTCTCTGGGAGG

       820                                 830
V  W  S  Y  G  E  R  P  Y  W  E  M  S  N  Q
TGATGTCTTATGGAGAGAGACCATACTGGGAGATGTCCAATCAG                    2592

                                    ----------------------------------
 D  V  I  K  A  V  D  E  G  Y  R  L  P  P  P  M  D  C  P  A  A
GATGTAATTAAAGCTGTAGATGAGGGCTATCGACTGCCACCCCCCATGGACTGCCCAGCTGCCT
```

FIG 1g

SUBSTITUTE SHEET

```
---------------------- 861
L  Y  Q  L  M  L  D  C  W  Q  K  D  R  N  N
TGTATCAGCTGATGCTGGACTGCTGGCAGAAAGACAGGAACAAC                    2700

      870                                880
 R  P  K  F  E  Q  I  V  S  I  L  D  K  L  I  R  N  P  G  S  L
AGACCCAAGTTTGAGCAGATTGTTAGTATTCTGGACAAGCTTATCCGGAATCCCGGCAGCCTGA

  890                                900
K  I  I  T  S  A  A  A  R  P  S  N  L  L  L
AGATCATCACCAGTGCAGCCGCAAGGCCATCAAACCTTCTTCTG                    2808

                   910                                920
 D  Q  S  N  V  D  I  S  T  F  R  T  T  G  D  W  L  N  G  V  R
GACCAAAGCAATGTGGATATCTCTACCTTCCGCACAACAGGTGACTGGCTTAATGGTGTCCGGA

                930
T  A  H  C  K  E  I  F  T  G  V  E  Y  S  S
CAGCACACTGCAAGGAAATCTTCACGGGCGTGGAGTACAGTTCT                    2916

940                                950                                960
 C  D  T  I  A  K  I  S  T  D  D  M  K  K  V  G  V  T  V  V  G
TGTGACACAATAGCCAAGATTTCCACAGATGACATGAAAAAGGTTGGTGTCACCGTGGTTGGGC

                         970
P  Q  K  K  I  I  S  S  I  K  A  L  E  T  Q
CACAGAAGAAGATCATCAGTAGCATTAAAGCTCTAGAAACGCAA                    3024
```

FIG 1h

```
                  980
 S    K    N    G    P    V    P    V    *
TCAAAGAATGGCCCAGTTCCCGTGTAAAGCACGACGGAAGTGCTTCTGGACGGAAGTGGTGGCT
GTGGAAGGCGTCAAGTCATCCTGCAGACAGACAATAATTCTGGA                    3132
```

FIG 1i

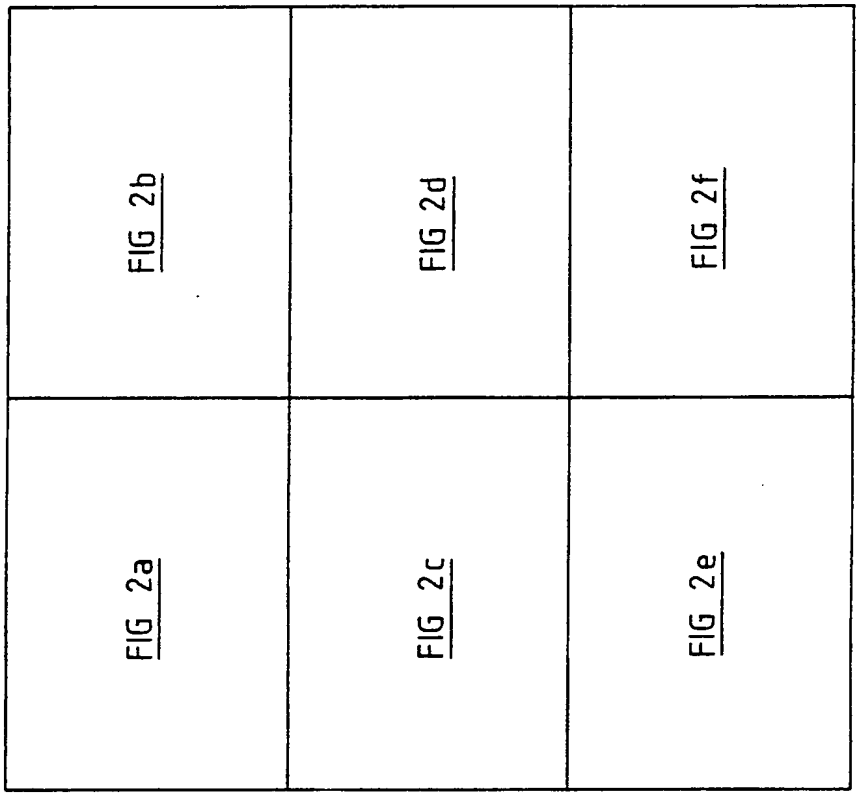


FIG 2

```
HEK  ..MDCQLSILLLLSCSVLDSFGELIPQPSNEVNLLDSTKIQGELGWISYPSHGWEEISGVDE

ELK  MAL--...L--F-LA-AVAAME-.........T-M-TR-ATA----TAN-AS----V--Y--


HEK  TFNLYYMESDDDHGVK....FREHQFTKIDTIAADESFTQMDLGDRILKLNTEIREVGPVNK

ELK  ------Y-T-SVIAT-KSAFWS-APYL-V----------S-V-F-G-LM-V---V-SF--LTR


HEK  SKEEDPP.RMYCSTEGEWLVPIGKCSCNAGYE.ERGFMCQACRPGFYKALDGNMKCAKCPPH

ELK  AE-V-V-IKL--NGD---M----R-T-K----P-NSVA-K--PA-TF--SQEAEG-SH--SN
```

FIG 2a

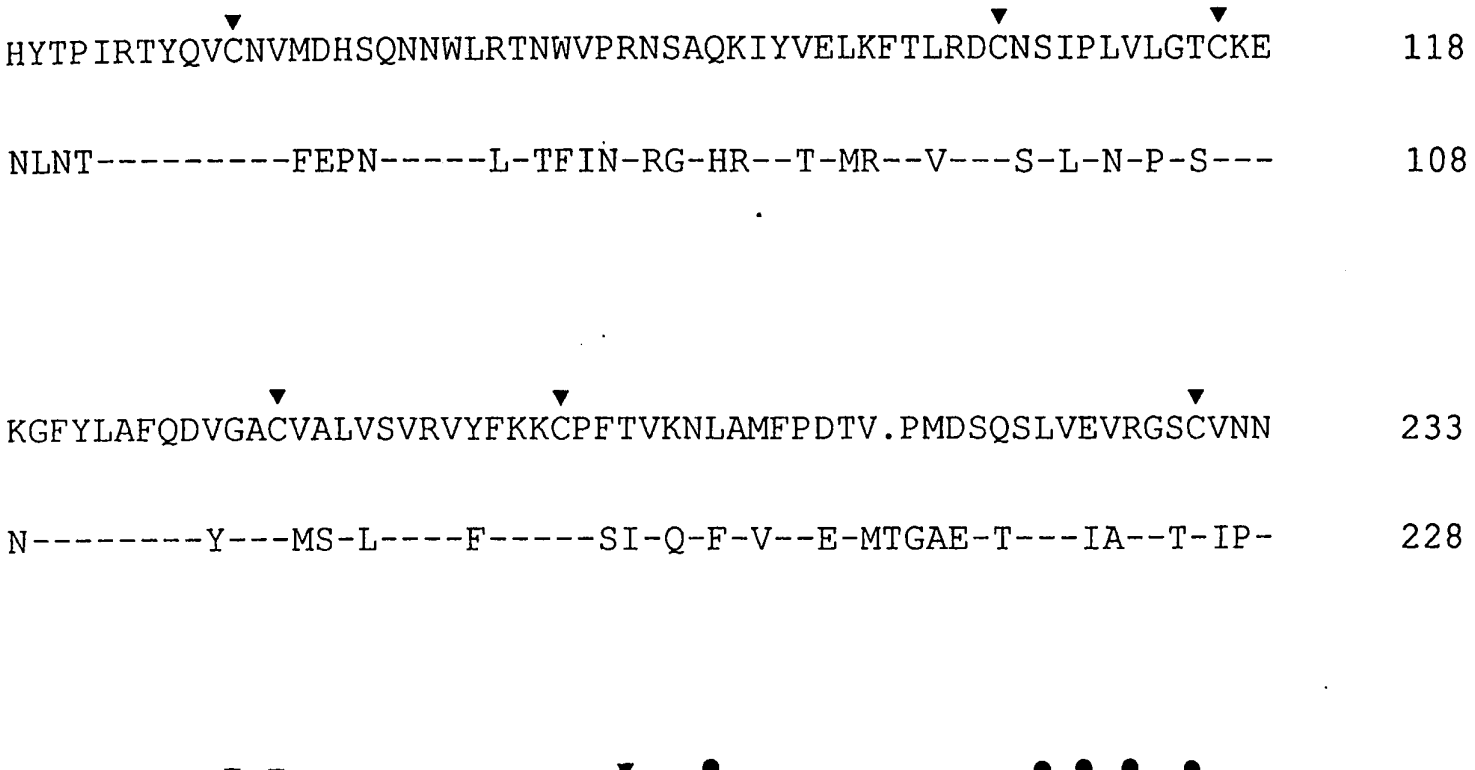


FIG 2b

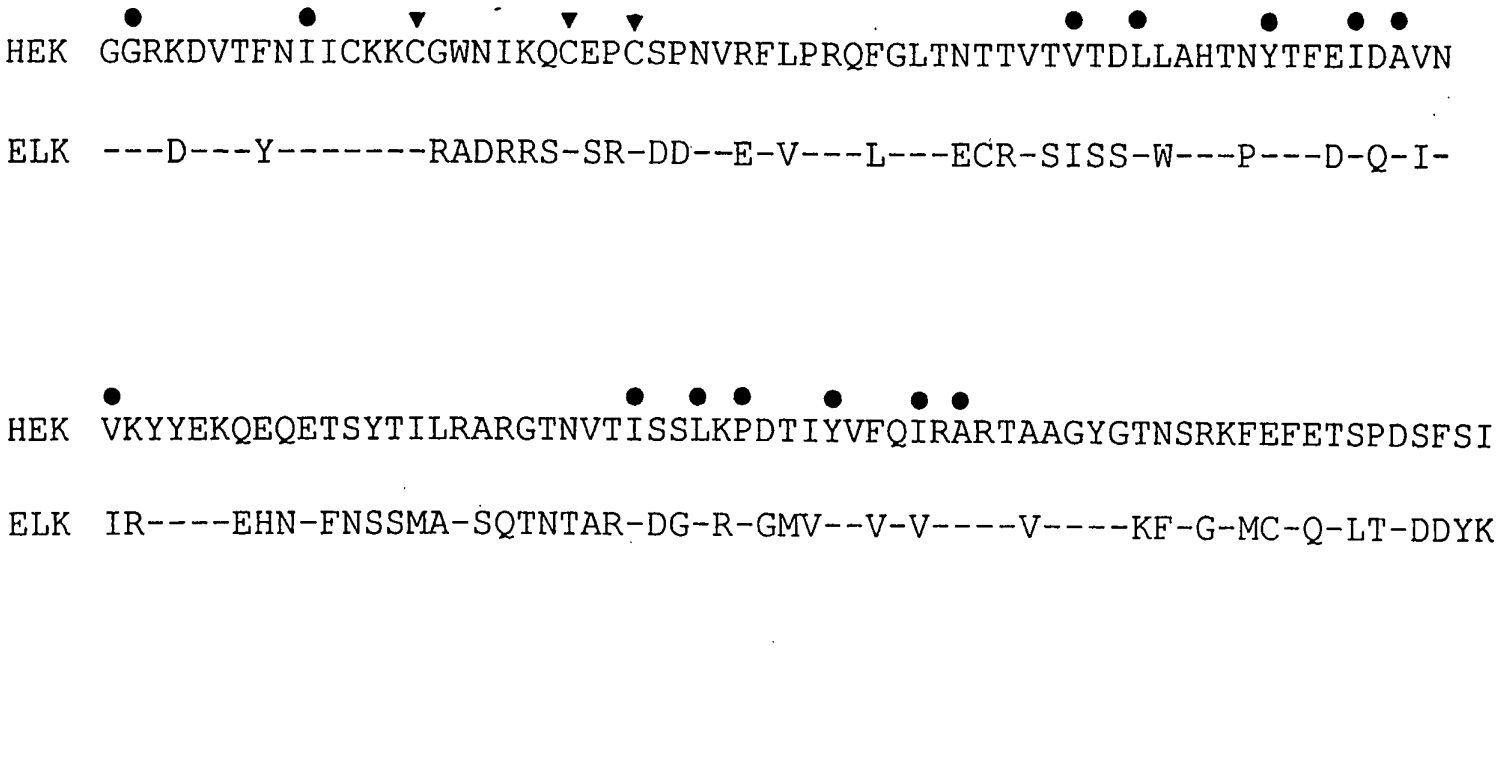


FIG 2c

```
                       ●               ● ● ●  ●    ●    ●
GVSELSSPPRQFAAVSITTNQAAPSPVLTIKKDRTSRNSISLSWQEPEHPNGIILDYE   471

---SK-PF-P-HVS-N---------T-PIMHQVSATMR--T---PQ--Q---------   468


SGESSQVVMIAISAAVAIILLT..VVIYVLIGRFCGYKSKHGADEKRLHFGNGHLK     589

-ELRE-LPL--G---AGVVFVVSL-A-SIVCS-KRA-SKEAVYSD-LQ-YST-RGS     588


IKTLKVGYTEKQRRDFLGEASIMGQFDHPNIIRLEGVVTKSKPVMIVTEYMENGSLDS   709

-----A--S--------S----------------------R----I--F----A---   707
```

FIG 2d

```
HEK  FLRKHDAQFTVIQLVGMLRGIASGMKYLSDMGYVHRDLAARNILINSNLVCKVSDFGLSRVLEDD.

ELK  ---QN-G---------------A------E-N------------V---------------Y-Q--T


HEK  MSNQDVIKAVDEGYRLPPPMDCPAALYQLMLDCWQKDRNNRPKFEQIVSILDKLIRNPGS

ELK  -------N-IEQD-------------H------------S--R-AE--NT---M----A-


HEK  TDDMKKVGVTVVGPQKKIISSIKALETQSKNGPVPV*

ELK  SE-LLRI---LA-H----L---HSMRV-MNQS-SVMA*
```

FIG 2e

```
PEAAYTTR.GGKIPIRWTSPEAIAYRKFTSASDVWSYGIVLWEVMSYGERPYWE    827

SDPT--SSL-----V---A-----------------------M-----F------D  827

LKIITSAAARPSNLLLDQSNVDISTFRTTGDWLNGVRTAHCKEIFTGVEYSSCDTIAKIS  947

--TVATIT-V--QP---R-IP-FTA-T-VD---SAIKMVQYRDS-LTAGFT-LQLVTQMT  947
```

FIG 2f

FIG 4

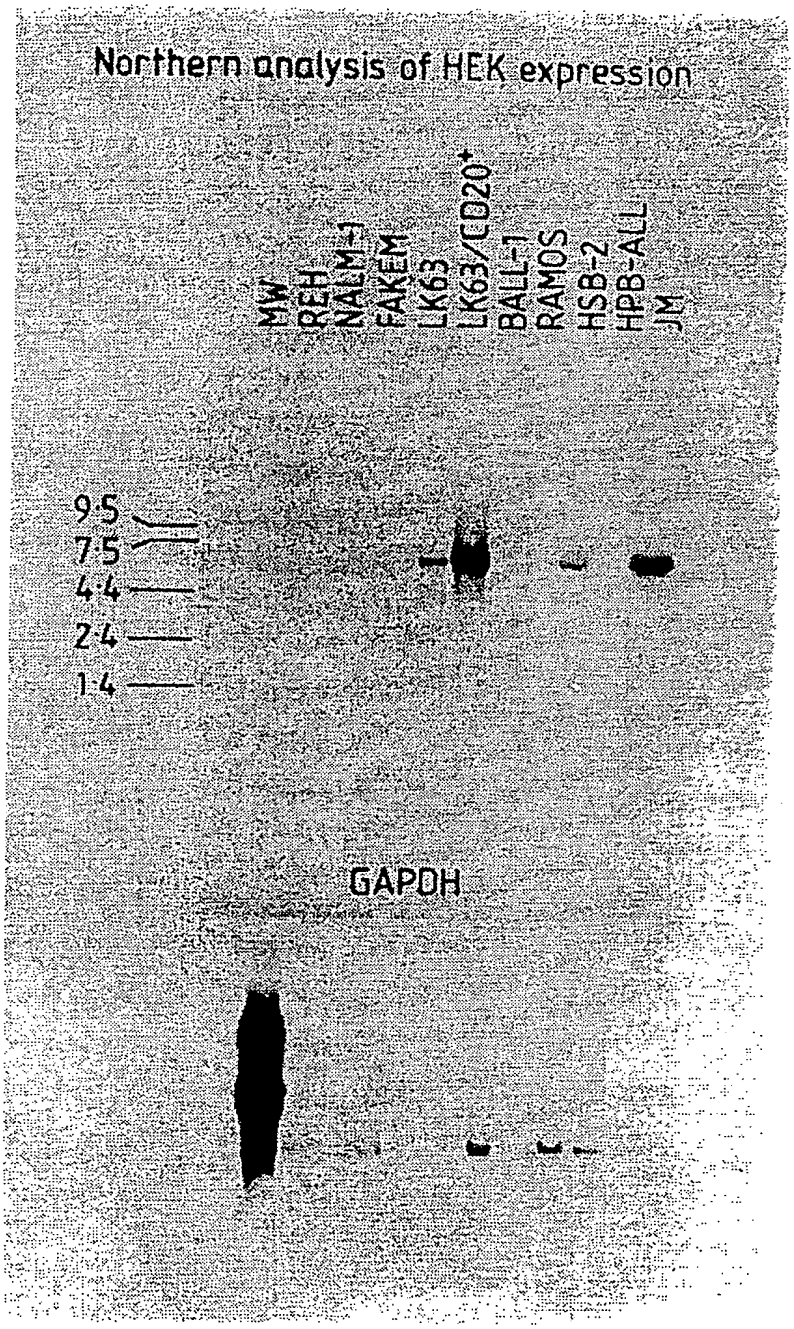


FIG 5

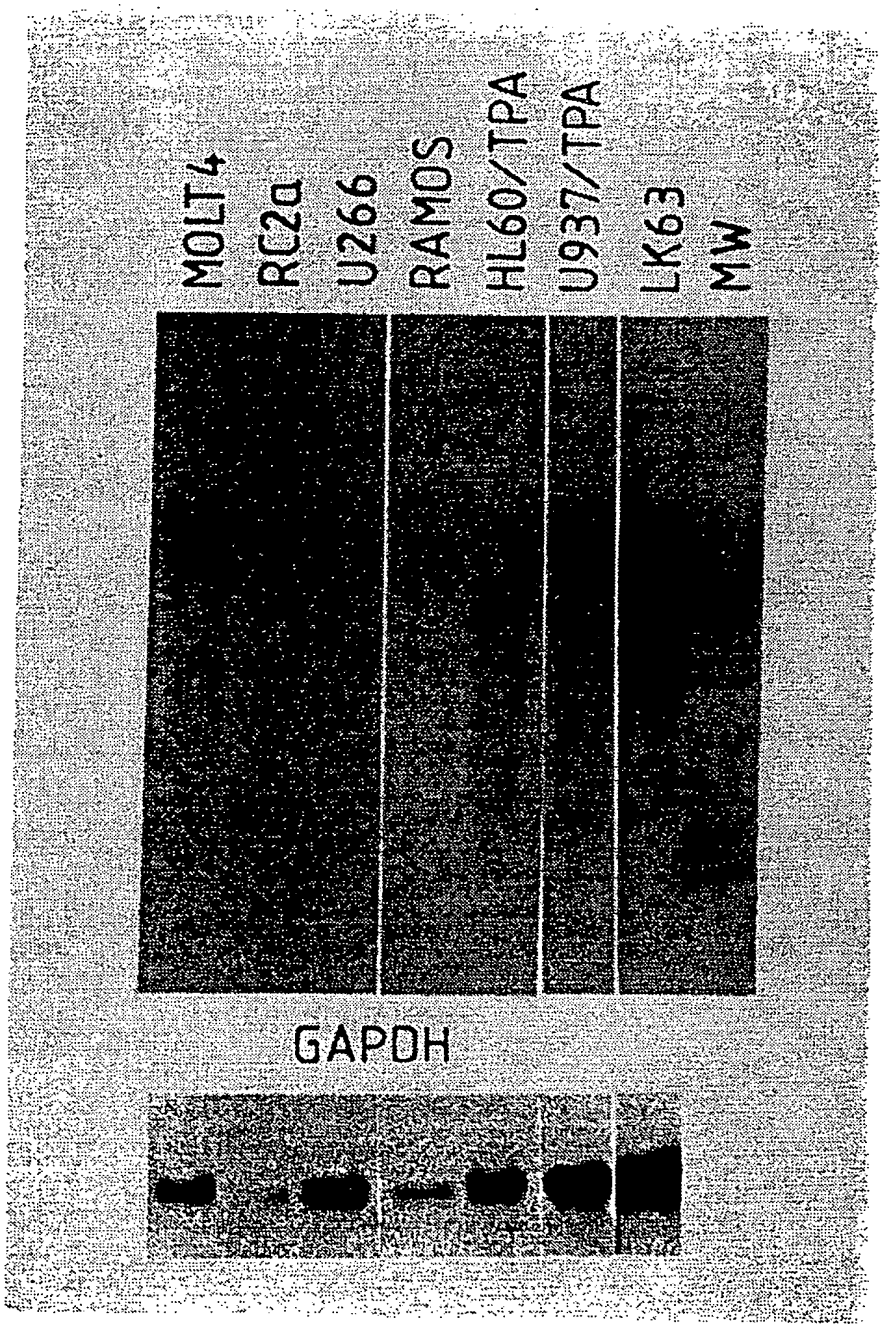
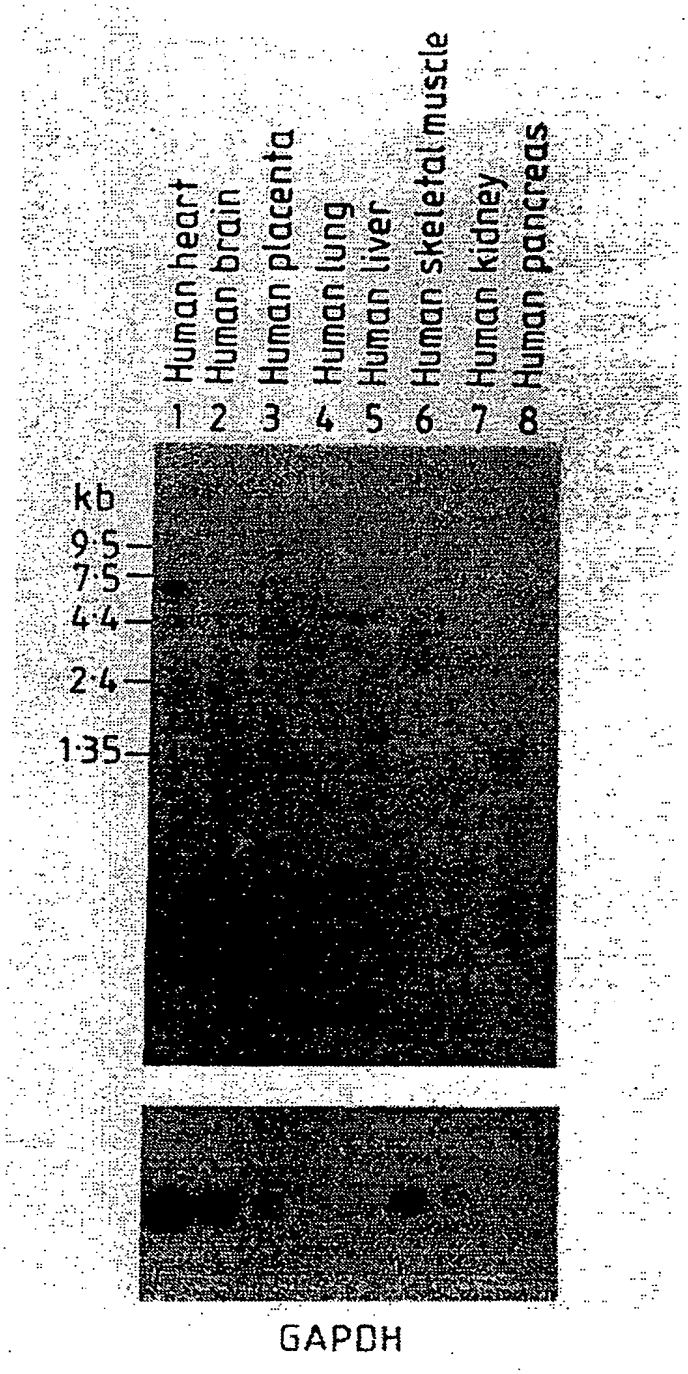


FIG 6


Human heart
Human brain
Human placenta
Human lung
Human liver
Human skeletal muscle
Human kidney
Human pancreas
1 2 3 4 5 6 7 8
kb
9·5
7·5
4·4
2·4
1·35
GAPDH

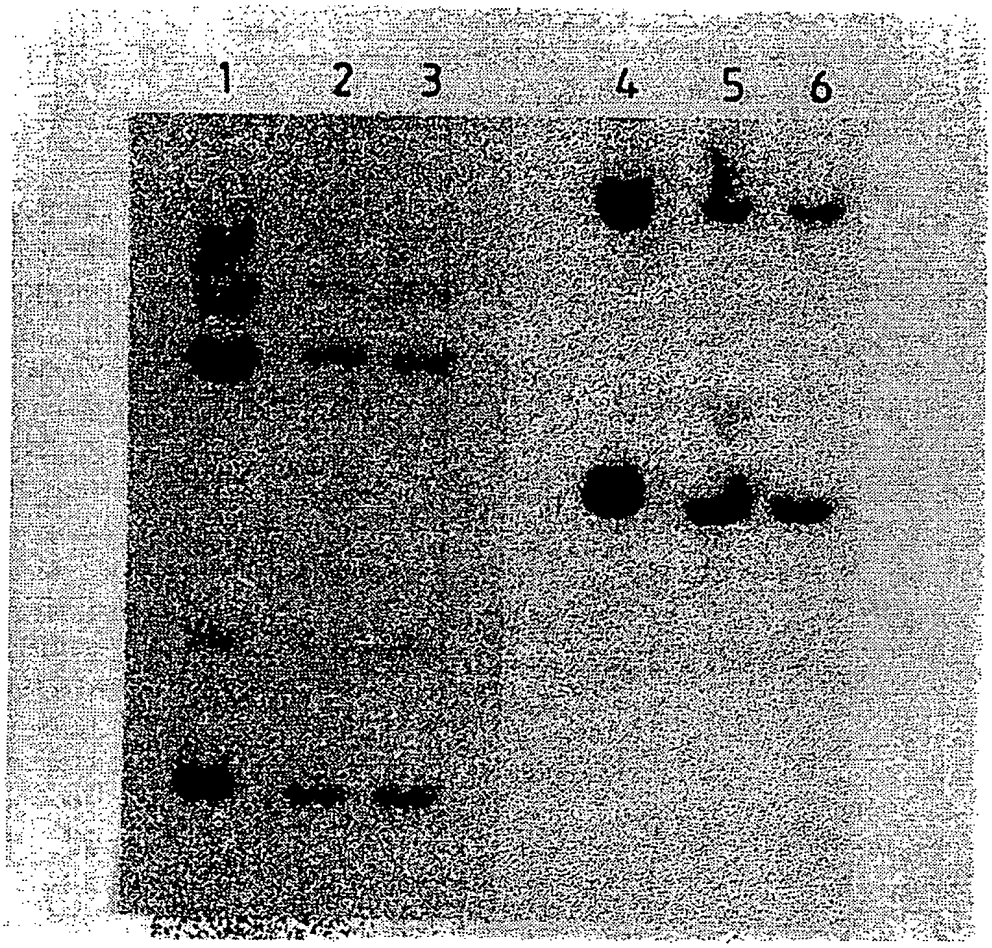
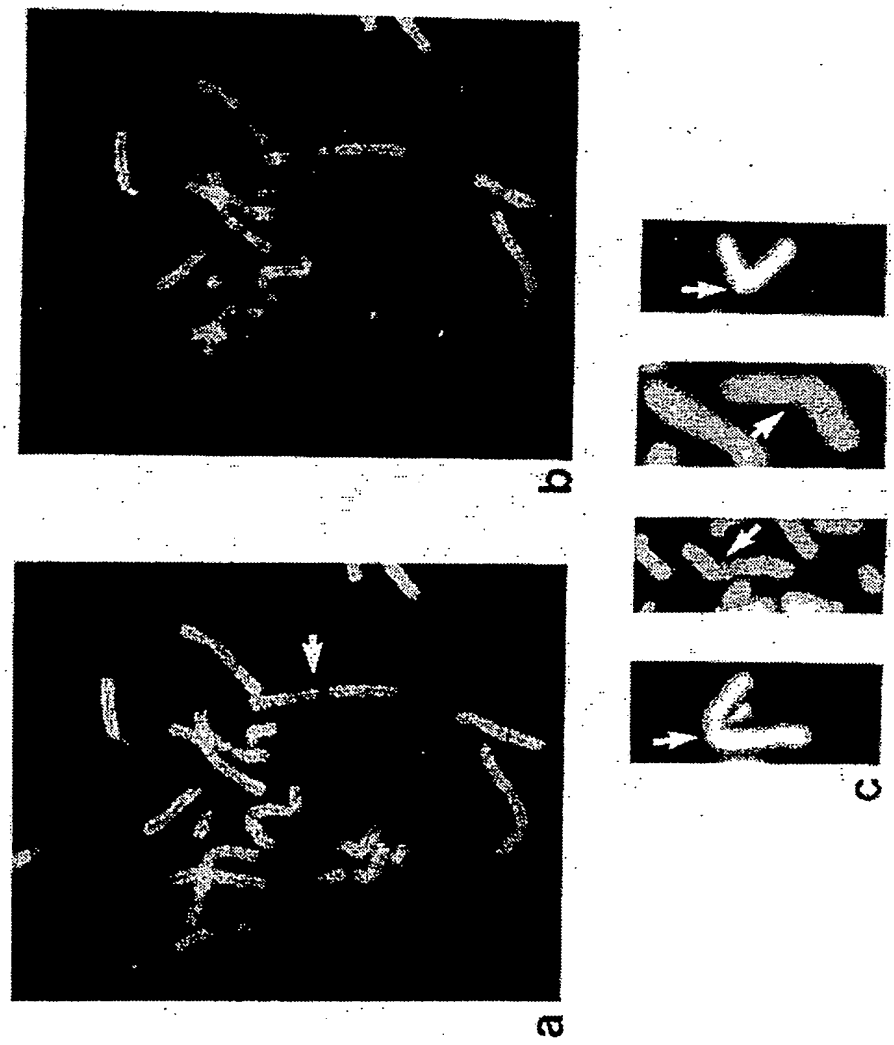


FIG 7

FIG 8



PROTEIN ANALYSIS: hek protein
Hydrophobicity of Kyte and Doolittle

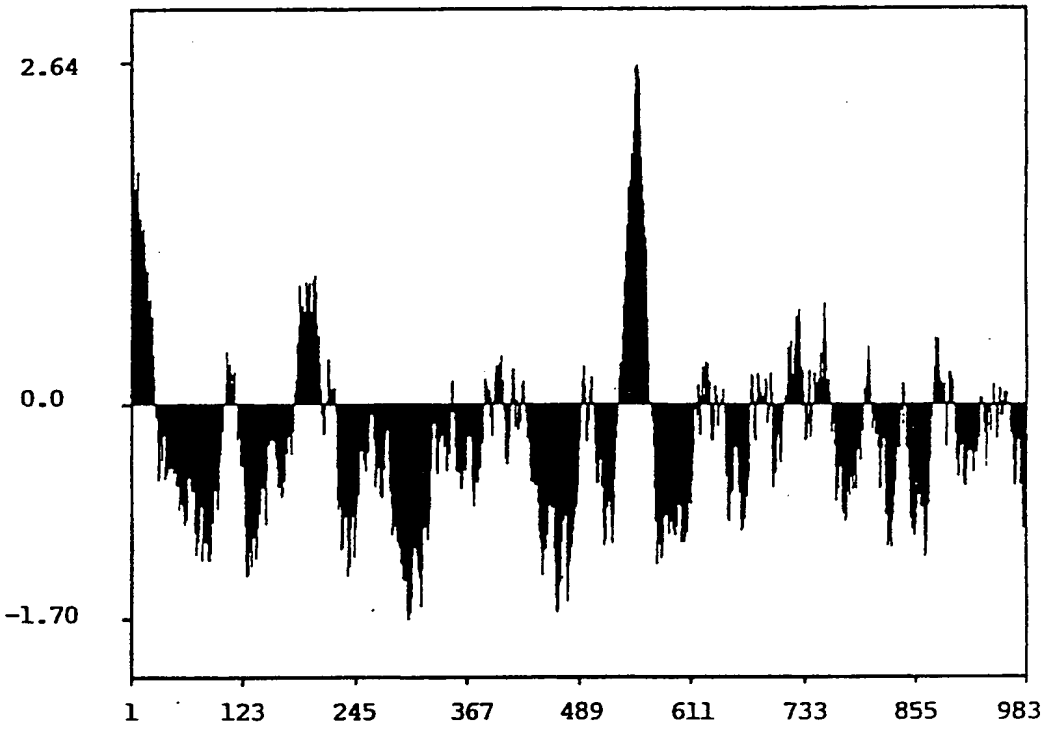


FIGURE 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU92/00294

A. CLASSIFICATION OF SUBJECT MATTER
Int. Cl.[5] C12N 9/12 15/54 A61K 37/52

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC : C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU : IPC C12N 9/12 15/54 A61K 37/57

Electronic data base consulted during the international search (name of data base, and where practicable, search terms used)
DERWENT DATABASE; WPAT, CHEM ABS KEYWORDS: TYROSINE KINASE BIOTECHNOLOGY ABSTRACTS: KEYWORDS AS ABOVE.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P, A	AU,A, 85169/91 (U.S. DEPARTMENT OF COMMERCE) 20 February 1992 (20.02.92) Claim 1.	1

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 September 1992 (29.09.92)	8 Oct 1992 (08.10.92)

Name and mailing address of the ISA/AU

AUSTRALIAN PATENT OFFICE
PO BOX 200
WODEN ACT 2606
AUSTRALIA

Facsimile No. 06 2853929

Authorized officer

G. Collins

Telephone No. (06) 2832082

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